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Induction by human serum of resistance to serum in *Neisseria gonorrhoeae:*

A clinical survey of patients with gonorrhoea

P M V MARTIN,* P V PATEL,† J C CLAY,‡ N J PARSONS,† AND H SMITH†

*From the *Unité d'Ecologie bactérienne, Institut Pasteur, Paris, France, the †Department of Microbiology, University of Birmingham, and the ‡Venereology Department, Birmingham General Hospital, Birmingham*

SUMMARY Serum from 74 female and 170 male patients with gonorrhoea and from 72 male and 123 female controls was tested for its ability to induce in gonococci resistance to complement mediated killing by human serum. We confirmed two findings of a previous survey: firstly, a higher precentage of serum samples from women suffering their first infection than from female controls induced high resistance; secondly, no serum sample taken from infected women with complications (mainly salpingitis) induced high resistance. The number of serum samples from female patients with repeat infection was too small for conclusions to be drawn. In men, however, there were no significant differences between patients and controls, or between patients with first or repeat infection, in the percentage whose serum induced high resistance. The pattern of these results on the induction of resistance to serum correlates with the general clinical aspects of gonococcal infections; namely, wide clinical differences in symptoms in women contrasting with a more uniform pattern in men.

Introduction

The resistance of *Neisseria gonorrhoeae* to complement mediated killing by fresh human serum is considered to be important in the pathogenesis of gonorrhoea.[1] In many strains of gonococci, this resistance is lost when they are subcultured in the laboratory.[1] We have been investigating the factors that induce resistance in vivo and their relevance to infection.[1] A laboratory cultured strain of *N gonorrhoeae* which was sensitive to serum, BS4 (agar), was initially induced to resist serum by growing it in plastic chambers implanted subcutaneously in guinea pigs; and, subsequently, by incubating it for three hours at 37°C in a medium containing guinea pig serum or its low molecular weight fractions.[1] Evidence that the induction of resistance may be important in human disease was provided by showing: (a) the presence in human serum of a resistance inducing factor similar to that of the guinea pig[2]; (b) the ability of male and female genital secretions to induce resistance to serum[3]; and (c) the ability of the low molecular weight fraction of guinea pig serum to induce resistance to serum in all of 30 recent isolates of *N gonorrhoeae*.[4] In a previous survey of correlations between the ability to induce resistance and the existence of gonococcal infections in 50 men and 50 women, there was no appreciable difference between serum samples taken from the patients and those from an equal number of controls.[5] Examination of the results of that study in relation to the type of gonococcal infection, however, showed: that serum from female patients with complications (salpingitis), or repeat infection, or both, did not induce resistance; that serum from female patients with their first gonococcal infection induced higher resistance in gonococci than serum from controls; and that serum from male patients with repeat infection induced higher resistance than serum from controls.[5] The limited number of serum samples examined in that study did not permit appreciable differences to be shown, except between the combined results from women with complications and those with repeat infections compared with the results from women with first infections.[5] We here report the results of a larger survey, which support two of the findings of the previous study.

Address for reprints: Professor H Smith, Department of Microbiology, South West Campus, P O Box 363, University of Birmingham, Birmingham B15 2TT

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Patients, materials, and methods

STUDY POPULATION

Blood samples were taken from 244 unselected patients (170 men and 74 women) attending the General Hospital, Birmingham, who underwent routine venepuncture on the day a first or subsequent attack of gonorrhoea was diagnosed, and from 195 healthy blood donors (72 men and 123 women) via the Regional Blood Transfusion Service, Birmingham. In all patients the initial diagnosis was made by examining Gram stained smears, and was confirmed by culture in each case. Salpingitis was diagnosed in women with pelvic pain and tenderness. The clinical findings were not confirmed by laparoscopy, and smears were not taken for culture of *Chlamydia trachomatis*. There were two groups of patients, those suffering a first infection, and those suffering a repeat infection. A third group among the women was those with complications (salpingitis, and in one woman additional perihepatitis). Two of the women with repeat infection also had complications, as did three in our previous report.[5]

TEST FOR INDUCTION OF RESISTANCE TO SERUM

The gonococcal strain BS4 (agar) and the methods of inducing resistance and measuring its extent were as described previously.[5 6] Serum was separated and all serum samples were tested twice after freezing and thawing as before.[2 5]

STATISTICAL ANALYSIS

Results were analysed by the χ^2 test, with Yates's correction for small numbers (less than 10).

Results

Table I shows the results of this study, which confirmed those of the previous report in two respects: a larger percentage of the serum samples that induced high resistance (that is, that converted more than 50% of the test gonococci to resistance) came from women with a first infection (28%) than from the female controls (20%). None of the six female patients who had complications had serum that induced high resistance. A larger percentage of serum samples that induced high resistance came from women with repeat infection (24%) than from the female controls (20%). This contrasted with the absence of such activity in the 11 samples from women with repeat infection in the previous survey.[5] Furthermore, a lower percentage of serum samples that induced high resistance came from men with a repeat infection (25%) than from men suffering their first infection (35%) and from control men (28%), again in contrast with the results of the previous survey.[5]

TABLE I *Ability of serum samples from 244 patients with gonorrhoea and 195 controls to induce resistance to serum in Neisseria gonorrhoeae (results from this survey)*

Origin of serum samples (No)	No (%) of samples inducing resistance in strain BS4 (agar) to fresh human serum	
	Low resistance (<50% of gonococci resistant)	*High resistance (>50% of gonococci resistant)*
All female patients (74)	55 (74)	19 (26)
First infection (53)	38 (72)	15 (28)
Repeat infection (17)	13 (76)	4 (24)
With complications* (6)	6 (100)	NIL
Female controls (123)	99 (81)	24 (20)
All male patients (170)	116 (68)	54 (32)
First infection (115)	75 (65)	40 (35)
Repeat infection (55)	41 (75)	14 (25)
Male controls (72)	52 (72)	20 (28)

*Mainly salpingitis. Two patients had repeat infections with complications.

Despite the support for two of the indications of the first survey, the relevant differences detected in the second survey were not in themsleves significant. Table II shows the combined results of the two studies. The percentage of serum samples inducing high resistance was significantly greater (χ^2 4·8, $p<0·05$) from female patients with first infections (31%) than from female controls (18%). None of the serum samples from 13 female patients with complications induced high resistance, which was significantly different (χ^2 5·4, $p<0·05$) from the 31% of samples inducing high resistance from female

TABLE II *Ability of serum samples from 344 patients with gonorrhoea and 295 controls to induce resistance to serum in Neisseria gonorrhoeae (combined results from this and previous survey[5])*

Origin of serum samples (No)	No (%) of samples inducing resistance in strain BS4 (agar) to fresh human serum	
	Low resistance (<50% of gonococci resistant)	*High resistance (>50% of gonococci resistant)*
All female patients (124)	93 (75)	31 (25)
First infection (88)	61 (69)	27 (31)
Repeat infection (28)	24 (86)	4 (14)
With complications* (13)	13 (100)	NIL
Female controls (173)	141 (81·5)	32 (18·5)
All male patients (220)	152 (69)	68 (31)
First infection (152)	103 (68)	49 (32)
Repeat infection (68)	49 (72)	19 (28)
Male controls (122)	90 (74)	32 (26)

*Mainly salpingitis. Five patients had repeat infections with complications.

patients with first infections, although the results for serum from controls (18%) was not significantly different (χ^2 2·9, p>0·05). As with results from the individual studies, the combined results for female patients with repeat infections (14% of serum samples inducing high resistance) showed no significant differences from the results of the other female groups; and there were no significant differences between the combined results of any of the male groups.

Discussion

The most important conclusion from the combined results of both surveys was the significant differences between the serum samples of the three groups of female patients, as well as between the group with first infections and controls. On the other hand, there was little difference in induction of resistance by serum between the male patients and the male controls or between the men with first and repeat infections. This correlates with the general pattern seen in clinics, with infection nearly always producing the same symptoms in men with urethral gonorrhoea, but either no symptoms or non-specific ones in women and frequently leading to complications.[7] It must be remembered, however, that the infection in men might display wider variation if more complete populations or different geographical locations were observed.

The combined results of the surveys show that a greater proportion of serum samples from female patients suffering their first infection had induced higher resistance than the serum of controls. It would be interesting to see if this correlation between infection and high resistance would be supported or strengthened by a similar study of the ability of genital secretions to induce resistance, because there was a greater proportion of active genital secretions than serum samples in previous work.[3]

A somewhat surprising result in the previous survey has been confirmed by this study. The serum from female patients with complications of gonococcal infection did not induce high resistance and, despite the small numbers of such patients (13 in all), this was significantly different from the results for serum samples from patients with their first infections. The fact that the difference in the combined results between serum from patients with complications and from controls is still not significant by the statistical analysis used, should not weigh heavily. Significant differences would almost certainly have been obtained if more serum from patients with complications had been examined because, in two independent surveys, no serum samples from the patients with complications induced high resistance, in contrast with 16% and 18% of the control serum sample. The possible reasons for this reverse correlation between complicated gonococcal infection and the presence of serum that induces high resistance have been discussed previously[5] and will be investigated in future work. Again, an examination of the genital secretions of such patients would be an interesting start to such investigations. The indication from the first study that there was also a reverse correlation in women patients between repeat infection and serum that induced high resistance was not confirmed by the second survey or by analysis of the combined results. In view of the diversity between the results of the two surveys in this respect, no conclusions could be drawn without conducting many more tests.

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